STRESS RESPONSES IN LAMBS TO DIFFERENT TAIL DOCKING METHODS

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SUMMARY

A study was conducted to assess the stress associated with different tail docking techniques using plasma cortisol concentrations as a physiological indicator, and to determine whether the responses are ameliorated by analgesic treatments. Tails were docked by the application of rubber Elastrator rings, the use of a sharp "cold" knife or a gas-heated "hot" knife. Analgesic treatments involved administration of epidural local anaesthetic or intramuscularly administered methadone.

There were significant increases ($P < 0.05$) in plasma cortisol concentrations in response to tail docking in the 60 minute period following the procedure. Furthermore, lambs tailed with the cold knife had significantly higher ($P < 0.05$) cortisol concentrations than lambs tailed with the other 2 techniques. The administration of methadone significantly lowered ($P < 0.05$) cortisol concentrations in contrast with epidural anaesthesia which failed to influence the cortisol responses to tail docking.

Keywords: tail docking, lambs, stress, cortisol, analgesia.

INTRODUCTION

It is standard practice in Australia to carry out certain animal husbandry practices, such as tail docking, castration, mulesing and dehorning, without anaesthesia or analgesia. These practices are generally recognised to cause some degree of stress and pain to the animals involved. With growing public awareness of animal welfare issues, there is increasing concern about whether these procedures cause undue stress. As a consequence there has been pressure to determine which of various alternative techniques available for certain management practices are preferable, and to examine the practicality and effectiveness of analgesic administration as a means of minimising the pain and stress associated with such practices.

Activation of the pituitary-adrenal axis is a widely recognized physiological response to stressors. A variety of stressful stimuli have been demonstrated to initiate pituitary corticotroph secretion (Selye 1973). In order to assess the activity of the pituitary-adrenal axis, circulating plasma concentrations of glucocorticoid hormones, such as cortisol, are often measured as an index of stress. Indeed, plasma cortisol has been widely used to quantify the stress associated with various sheep husbandry practices, from the effects of different handling and management practices (Kilgour and de Langen 1970; Purchas 1973), to electroimmobilisation (Jephcott *et al.* 1986) and routine surgical procedures with lambs (Shutt *et al.* 1987, 1988; Mellor and Murray 1989; Wood *et al. 1991).*

As part of a project to characterise pain and stress in lambs subjected to different methods of tail docking and to determine whether the pain and stress can be ameliorated by epidural anaesthesia or opioid analgesia, this study examined the physiological response to these procedures as indicated by changes in plasma cortisol concentrations.

MATERIALS AND METHODS

Preparation of animals

Prior to undertaking any studies it was essential to identify each individual ewe/lamb pair so that they could be kept together throughout the study period. This identification procedure was carried out at least a week prior to the study.

It was also necessary, on a daily basis for the week leading up to the study, to familiarise the animals to the shed environment where the study was to be undertaken, and more importantly to condition the lambs to human presence and to being handled and restrained. The potential stress and accompanying elevated blood cortisol levels associated with handling restraint and subjection to an unfamiliar environment (Kilgour and de Langen 1970) could otherwise influence the results of the study.

Experimental protocol

A total of 60 crossbred (Poll Dorset x Merino) and purebred Merino mixed sex lambs aged 3-5 weeks and with a mean liveweight of 11.2 ± 0.3 (sem) kg were used.

Twenty lambs were randomly divided into 4 groups of 5 with each group allocated 1 of 4 treatments;

tails left undocked (controls), tails docked surgically using a sharp "cold" knife, tails docked using a gas-heated "hot" knife (Brog Gas Detailer, Victoria, Australia) or tails fitted with rubber Elastrator rings (Elastrator Pty Ltd, Victoria, Australia). All docking procedures were carried out by commercially used methods at the third palpable joint of the tail. The lambs were restrained for a period of 2 minutes in a rotating mulesing cradle using simple hind leg restraints to hold them on their backs at an inclined position whilst the treatments were performed. The control group underwent the same handling procedures in the cradle as the treated groups.

Samples of blood (3 mL) were collected through indwelling jugular catheters at -30, -15, 0, 15, 30, 45, 60, 90, 120 and 180 minutes relative to treatment and placed into EDTA tubes. Each sample was immediately centrifuged and the plasma removed and stored frozen until assayed for cortisol concentration. The jugular catheters (18 G x 5 cm, Terumo Corporation, Tokyo, Japan) were inserted at least 6 hours prior to the experiment to allow the lambs to settle down before sampling, thereby ensuring stable baseline cortisol concentrations. In the time between collection of blood samples, the lambs were allowed to remain quietly in a pen with their mothers.

A further 20 lambs were randomly divided into 4 groups of 5 and subjected to the same treatment protocol described above. These groups, however, each received a pre-treatment of methadone (methadone hydrochloride 10 mg/mL, Parnell Laboratories, Sydney, Australia) (1 .0 mg/kg liveweight) administered intramuscularly into the upper hind leg 15 minutes prior to tail docking treatment. This dosage was selected after a methadone dose-response trial was undertaken to ensure an adequate concentration of methadone was present in the blood over the study period.

A final 20 lambs were also assigned to 4 random groups and underwent the same tail docking treatment protocol. These groups were given an epidural administration of 1.2 mL of 2% lignocaine containing adrenalin injected through the intervertebral space between the first and second coccygeal vertebrae 30 minutes prior to tail docking. This time was chosen to ensure maximal effects of the treatment at the time of tailing. Lambs weighing more than 15 kg received a larger dose of 1.5 mL.

Plasma cortisol concentrations were measured by radioimmunoassay. The antibody used had negligible cross-reactivity with corticosterone, deoxycorticosterone, progesterone, pregnenolone or dexamethasone. The sensitivity of the assay was 7.2 nmol/L, while between assay and within assay variation were 7% and 4% respectively.

Analysis of results

Plasma cortisol concentrations were expressed as the mean ± sem for the 5 lambs in each treatment group. The 3 pre-treatment sample concentrations (-30, -15, 0 minutes) were used to calculate a mean basal cortisol concentration for each group. Inspection of the resultant profile of cortisol concentration over time showed that peak plasma cortisol concentrations occurred within 60 minutes after docking. One-way analyses of variance were therefore conducted covering time periods 15, 30, 45 and 60 minutes to test for significant differences between the means of tail docking treatments and analgesic treatments respectively. A post-hoc analysis (Tukey) was then undertaken to carry out simultaneous pairwise comparisons between the mean cortisol concentrations over this 60 minute time period.

RESULTS

Tail docking technique

The mean cortisol concentrations for each tail docking treatment group over the 60 minute post tail docking period, together with the basal and 180 minute cortisol concentrations, are reported in Table 1. Maximum plasma cortisol concentrations occurred 30 minutes after tail docking in each treatment group. All tail docked groups had significantly increased ($P < 0.05$) plasma cortisol concentrations relative to the control group and to basal concentrations. Furthermore, lambs tailed with the cold knife had significantly higher ($P < 0.05$) cortisol concentrations than lambs tailed with the other 2 tail docking methods. Cortisol concentrations had returned to basal levels by 180 minutes after tail docking in most treatment groups.

Analgesic pre-treatment

The mean cortisol concentrations for the tail docking treatments under different analgesic regimes are reported in Table 1. A significant difference ($P < 0.001$) in plasma cortisol concentrations was seen between analgesic treatments. The administration of methadone curtailed the cortisol response to tail docking during the 60 minute period after docking. At this time cortisol concentrations were significantly lower ($P < 0.05$) for each of the methadone-treated tail docked groups compared to the corresponding groups receiving no analgesic pre-treatment. There was no significant difference between the tail docking techniques themselves with methadone pre-treatment.

By contrast, the epidural administration of local anaesthetic did not reduce the cortisol response to tail docking. Tail docked groups had significantly increased ($P < 0.05$) plasma cortisol concentrations in the 60 minute period after docking, relative to the local anaesthetic control group and to basal concentrations. Lambs tailed with the cold knife had significantly higher ($P < 0.01$) cortisol concentrations compared to the ring and hot knife treatment groups, which were not significantly different from each other.

Table 1. Mean plasma cortisol concentrations (nmol/L) (sem) in lambs undergoing various tail docking treatments under different analgesic regimes, over the 30 minute pre-treatment period, and during the initial 60 minute period and at 180 minutes following tail docking

	Time (mins)	Control	Rings	Cold knife	Hot knife
	-30 – 0	17.9 (3.7)^{a}	14.0 (1.7)^{a}	24.5 (3.0)^{a}	19.7 (2.2)^{a}
No analgesia	15 – 60	18.6 (2.8)^{a}	65.1 (7.9)^{b}	116.8 (10.8)^{c}	72.4 (9.3)^{b}
	180	6.2 (2.2)^{a}	26.3 (7.4)^{a}	53.4 (15.2)^{b}	22.7 (6.8)^{a}
	-30 – 0	23.8 (2.8)^{a}	27.3 (4.2)^{a}	23.9 (3.1)^{a}	29.8 (4.2)^{a}
Methadone	15 – 60	7.1 (1.1)^{a}	19.6 (2.9)^{a}	24.5 (5.3)^{a}	25.8 (4.8)^{a}
	180	15.4 (5.3)^{a}	14.4 (2.2)^{a}	85.0 (27.1)^{b}	20.3 (5.3)^{a}
	-30 – 0	41.2 (3.1)^{a}	45.8 (6.1)^{a}	41.8 (5.4)^{a}	47.2 (7.4)^{a}
Epidural anaesthesia	15 – 60	38.4 (4.5)^{a}	102.3 (9.1)^{b}	169.7(15.3)^{c}	75.8 (5.4)^{b}
	180	19.8 (5.7)^{a}	23.7 (7.9)^{a}	90.9 (22.5)^{b}	22.9 (9.3)^{a}

Means with different superscript letters are significantly different ($P < 0.05$).

DISCUSSION

The results of this study demonstrate substantial increases in plasma cortisol concentrations in response to tail docking for a period of up to 60 minutes. In addition, the magnitude of this response appears to differ between various tail docking techniques.

The significant cortisol response induced by the use of the cold knife suggests that this method of tailing results in a marked physiological stress response, presumably due to the tissue damage associated with tail removal. This is consistent with similar studies by Shutt *et al.* (1987, 1988) which reported plasma cortisol concentrations to be increased substantially in response to surgical tailing, to levels greater than those seen with the application of rings. However, in the present study, and in contrast to the cold knife results, the use of the hot knife (which is also a surgical method) did not produce a similarly pronounced cortisol response. Tail docking with rings, which causes tissue ischaemia, also did not produce such a marked cortisol response (Table 1). However, behavioural observations (data not shown) did indicate that the ring treatment resulted in more agitated and restless behaviour in lambs. Other studies have also concluded that, based on behavioural observations, the application of rings is particularly painful (Kuchel *et al.* 1989; Mellor and Murray 1989). This variability in cortisol response cautions against the use of plasma cortisol concentrations alone, as an indicator of stress, and suggests that other parameters, both behavioural and physiological, may also need to be used to fully assess stress responses associated with animal husbandry practices.

Epidural anaesthesia failed to reduce the cortisol responses to the tail docking treatments (Table 1). These results contradict the findings of Wood *et al.* (1991), who concluded that epidural anaesthesia significantly reduced the cortisol response to tail docking. However, that study was carried out on lambs less than 1 week old and assessed tail docking with rings only. The inability of the anaesthetic to reduce the cortisol response in the present study may be due to several factors including insufficient dosage, misplacement of the dose or the possibility of more than one pathway being responsible for instigating cortisol release. The physical restraint required during the administration on the epidural anaesthetic may explain the higher basal cortisol concentrations of those treatment groups.

The administration of methadone as a possible analgesic treatment modulated the cortisol responses in tailed lambs (Table 1). However, recent studies by Kuchel and Grant (unpublished) suggest that methadone provides only minimal effective analgesia in lambs. This implies that the hypothalamic-pituitary-adrenal axis may itself be affected by the methadone treatment, and that the resultant cortisol profile may not truly represent the "pain" response perceived by lambs.

There is increasing evidence that the secretion of endorphins, concomitantly released with cortisol (Mains *et al.* 1977), may provide some degree of inherent stress-induced analgesia which is beneficial for the animal and which may be responsible for adaptive pain suppression (Wolfe and Liebeskind 1983). Shutt *et al.* (1987, 1988) found significant increases in plasma β-endorphin levels in association with the increased cortisol concentrations observed after surgical tailing and other surgical procedures, and it will be important to examine β-endorphin concentrations in the samples obtained in the present study.

If sheep do use endogenous stress-induced analgesia mechanisms then surgical tailing may in fact be the preferable method. Certainly from a practical viewpoint the cold knife method is more appealing as the wound is faster to heal than with the other techniques (Bell 1987; Woods and Johnson 1988). If mulesing is being carried out along with tail docking then the hot knife method may be more appropriate as it reduces the loss of blood. Either way further work is clearly needed to investigate inherent stress-induced analgesic systems in lambs and to explore more practical means of analgesia for tail docking and other husbandry procedures.

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